## EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES

(11-17239)

## Summary sheet of validation data for a diagnostic test

The EPPO Standard PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* describes how validation should be conducted. It also includes definitions of performance criteria.

| Target Organism  | Bursaphelenchus xylophilus   |                        |  |
|--|--|------------------------|--|
| Short description  | Detection of Bursaphelenchus xylophilus in wood extract with real-time PCR Leal et al. 2005  |                        |  |
| Laboratory contact details   | Anses, Laboratoire de la Santé des<br>Végétaux - Unité de Nématologie<br>Domaine de la Motte au Viconte BP<br>35327, 35653 Le Rheu, France   |                        |  |
| Date and reference of the validation report                                | 2011-02 - Anses 2011 Rapport<br>d'évaluation d'outils moléculaires de<br>détection de Bursaphelenchus xylophilus<br>sur extrait de bois  |                        |  |
| Validation process according to EPPO Standard PM 7/98:                     | Yes  |                        |  |
| Reference of the test description  | 0<br>Leal I, Green M, Allen E, Humble L, Rott M<br>(2005) An effective PCR-based diagnostic<br>method for the detection of<br>Bursaphelenchus xylophilus in wood<br>samples from lodgepole pine. Nematology<br>Vol.7(6), 833-842 |                        |  |
| Is the test the same as described in the EPPO DP?                          | No<br>Other test   |                        |  |
| Is the lab accredited for this test?                                       | No   |                        |  |
| Plant species tested (if relevant)   |  |                        |  |
| Matrices tested (if relevant)  | Wood extract   |                        |  |
|  |  |                        |  |
| List of methods used   |  |                        |  |
| Method for extraction / isolation / baiting of target organism from matrix |  |                        |  |
| Molecular methods, e.g.<br>hybridization, PCR and real time PCR            | Х  | real time PCR (Taqman) |  |
|  |  |                        |  |

| Serological methods: IF, ELISA,<br>Direct Tissue Blot Immuno Assay  |   |  |  |  |
|---|---|--|--|--|
| Plating methods: selective isolation  |   |  |  |  |
| Bioassay methods: selective enrichment in host plants, baiting, plant test and grafting.                                      |   |  |  |  |
| Pathogenicity test  |   |  |  |  |
| Fingerprint methods: protein profiling, fatty acid profiling & DNA profiling  |   |  |  |  |
| Morphological and morphometrical methods intended for identification  |   |  |  |  |
| Biochemical methods: e.g. enzyme electrophoresis, protein profiling   |   |  |  |  |
| Other   |   |  |  |  |
| Analytical sensitivity (= limit of detection)   |   |  |  |  |
| What is smallest amount of target that can be detected reliably?  | one nematode  |  |  |  |
| <u>Diagnostic sensitivity</u>   |   |  |  |  |
| Proportion of infected/infested samples tested positive compared to results from the standard test, see appendix 2 of PM 7/98 | 100%  |  |  |  |
| Specify the standard test   | morphology  |  |  |  |
| Analytical specificity  |   |  |  |  |
| Specificity value   | 100%  |  |  |  |
| Number of strains/populations of target organisms tested  | 7 populations (see table1)  |  |  |  |
| Number of non-target organisms tested   | 15 populations (see table1)   |  |  |  |
| Cross reacts with (specify the species)   | none  |  |  |  |
| <u>Diagnostic Specificity</u>   |   |  |  |  |
| Proportion of uninfected/uninfested samples (true negatives) testing negative compared to results from a standard test        | 99% (6 false positives results/490 uninfested samples and 1 false negative result /13 infested samples) |  |  |  |
| Specify the standard test   | morphology  |  |  |  |
| Reproducibility   |   |  |  |  |
|   |   |  |  |  |

| Provide the calculated % of agreement for a given level of the pest (see PM 7/98)  | 100% for one single nematode   |  |  |
|--|--|--|--|
| Repeatability  |  |  |  |
| Provide the calculated % of agreement for a given level of the pest (see PM 7/98)  | 100% for one single nematode   |  |  |
| <u>Test performance study</u>  |  |  |  |
| Test performance study?  | No   |  |  |
| Include brief details of the test<br>performance study and its output.It<br>available, provide a link to published<br>article/report |  |  |  |
| Other information  |  |  |  |
| Any other information considered useful e.g. robustness, ease of performing the test, etc.   | The full report is available upon request to the laboratory  |  |  |
| •  |  |  |  |
| The following complementary files are available online:  | <ul> <li>Table 1 List of species and population tested</li> <li>Table 2 comparison of different PCR tests B xylophilus identification</li> </ul> |  |  |